UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

002021

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM 23 JUN 1982

Caswell # 125 D

TO: Jackie Dziuban
PM Team No. 25
Registration Division (TS-767)

SUBJECT: Studies submitted by Ciba-Geigy on Terbutryn in response to RPAR.

Four studies were received from Ciba-Geigy (Date of submission; 4/23/82; Accession numbers 247365-247368) in response to FIFRA Section 6 (a) (2). Toxicology Branch has reviewed the studies, and the conclusions are as follows:

Conclusions and Recommendations

(1) Rat intrasanguine host - mediated assay using S. typhimuruim (Ames) to test for the mutagenicity of GS-14260. This test showed no mutagenic effects but does not meet minimum criteria set by EPA standards.

(2) In vivo hamster cytogenetic study on bone marrow cells showed that GS-14260 caused no chromosome aberrations in this test. Meets minimum criteria set by EPA standards.

(3) Chromosome studies in the germinal epitheluim of male mice suggested that GS-14260 caused no chromosome aberrations in spermatogenia, but the study is only of supplementary value and does not meet minimum EPA standards.

(4) Dermal absorption study using ^{14}C-Terbutryn applied to rat skin. Invalid. All supporting experimental results are requested.

1 D 4

Reviews

1. Host-mediated assay. A rat intrasanguine host-mediated assay using Salmonella typhimurium (Ames) to test for the mutagenicity of GS-14260 (Terbutryn technical) was performed by Ciba-Geigy, Basle, Switzerland, authored by P. Arni and D. Miller, dated 11/20/81. Male albino rats (20-41g; 6 mice per group) that had been fasted for 16 hours were administered Terbutryn in CMC by gavage at levels of 0, 500, 1000 or 2000 mg/kg at 2 hour, 1 hour and immediately before the injection of about 10^{10} bacteria (TA 1535, 1537, 98, or 100) into the tail vein. One hour after injection the rats were sacrificed, and the bacteria recovered from the livers. 10^{7} to 10^{8} bacteria were recovered from each host. 0.2 ml of this suspension was spread on each of 5 plates. The mutation rate (reversion from histidine auxotropy to prototrophy) was not increased in strains TA 1535, 1537 and 98 by administration of Terbutryn, but mutagenic effects were observed in strain TA 100. However, in two replicate experiments no mutagenic effects were seen in TA 100, so the mutagenic effect was not confirmed.

There are a few defects in this study. No postitive controls were mentioned, and this is a serious omission in the host-mediated assay. Positive controls could be performed on 1) the bacteria before, or 2) after injection and recovery from the host, or 3) the host itself by injection of the positive control chemical into the host, followed by injection and recovery of the bacteria. The mutagenic effects of the positive control chemical on the bacteria are then scored by the normal procedure for an Ames' test. Performance of positive control #3 would have been sufficient.

Terbutryn was administered beginning at 2 hours before injection of the bacterial tester strains, and the animals were sacrificed 1 hour after injection of the bacteria. However, the authors did not prove, or even state, that the selected times of administration of test substance and tester strains were the optimal times.

Conclusion:

This study suggests that GS-14260 caused no mutagenic effects, but because of protocol limitations this study does not meet minimum criteria set by EPA standards.

2

2. In vivo hamster cytogenetics test. Test performed by G. Hool, E. Puri, and D. Muller of Ciba-Geigy, Basle, Switzerland, dated 11/24/81. Chinese hamsters (21-33g) were administered 0, 750, 1500, or 3000 mg/kg of GS 14260 by gavage on each of 2 consecutive days. Four animals per sex per dose were used, and 6 animals per sex were used in the positive and negative control groups. The animals were injected i.p. with 10 mg colcemide/kg 2 hours after the second dose and sacrificed by cervical dislocation 4 hours later. Chromosome preparations were made from bone marrow cells. Two animals per sex per dose were evaluated for chromosome aberrations. One hundred metaphase plates from each animal were analyzed. In two animals in the control groups (0.5% MC + 0.1% Tween 80) an acentric fragment was observed. The test groups showed the following chromosome aberrations: low dose: one break; intermediate dose: one minute chromosome; high dose; none. Cyclophosphamide (64 mg/kg) administered as positive control resulted in 22.0% with chromatid aberrations, 11.8% with chromosome aberrations, and 0.25% of the cells scored revealed pulverizations. Thus GS 14260 was not mutagenic in this test.

Conclusion: GS 14260 caused no chromosome aberrations in Chinese hamsters in this test. Meets minimum criteria set by EPA standards.

3. Chromosome ~~Chromic~~ studies in male germinal epithelium. This study is dated 11/23/81; directed and reviewed by G. Hool and D. Muller, respectively, of Ciba-Geigy, Basle, Switzerland. An NMRI-derived strain of male mice (15 animals per test group, 12 animals in the control group) were administered GS 14260 by gavage (486 and 1458 mg/kg in 20 ml/kg 0.5% CMC--CMC alone was administered as negative control) on 5 consecutive days (days 0-4). The authors state that 4,370 mg/kg was the LD_{50}. On day 5 the mice were given 10 mg/kg Colcemide and sacrificed three hours later. 100 metaphase figures from the testes of each of 8 animals in each group were scored for chromosome aberrations. One animal in the control, and the high dose, group showed one gap; otherwise no chromosome aberrations were observed. Thus no mutagenic effects on spermatogonia were observed.

This study attempts to determine whether or not Terbutryn has heritable mutagenic effects, but is only of supplementary value for the following reasons. Lack of a positive control. Although it may not be necessary to perform a positive control (e.g. mitomycin c, 6-mercaptopurine) during each test series, at least the authors could have provided evidence to show that this procedure as performed in their lab is sensitive to known mutagens.

3

Lack of experimental detail, particulary in the isolation and preparation of the germ cells for scoring. This test has not been standardized as much as some of the other tests for heritability, and as such needs to be reported more completely. No data presented. Under "Results" only a summary sentence appears in the text, and no Tables or Figures are included. What types of "chromosome and chromatid aberrations" were scored.?

Conclusion:

No chromosome aberrations observed, but without further data the study does not meet minimum criteria set by EPA standards and is only of supplementary value.

4. Dermal absorption of ^{14}C-Terbutryn by rats. Submitted by B. Simoneaux of Ciba-Geigy, Greensboro, N.C.; issue date 4/15/82, Report No. ABR-82016.

This study is inadequately reported, and should be considered invalid until supporting experimental data is received by EPA. There are some important questions that must be answered. For example: (1) How many animals comprised each data point? (2) Please explain the quench correction procedure, i. e., in converting cpm to dpm to ug. (3) Did they (the experimenters) distinguish between "on" as apposed to "in" the skin? The report mentioned in the Methods section that ^{14}C in the acetone washes was counted separetely from that remaining in the washed, excised skin, but the data is not listed under Results and Discussion.

In addition, the following information would be useful to EPA in evaluating this study if the data were obtained. (1) It is unfortunate that the study was termnated at 1 day, particuilarly since ^{14}C-was still being taken up from the skin at 24 hours. In this regard, RBC's appeared to absorb radioactivity throughout the observation period, and one wonders whether or not Terbutryn binds to the RBC. (2) Does Terbutryn bind to fat? Terbutryn is not very soluble in water (about 58 ppm), but very soluble in organic solvents, and one wonders whether it might accumulate in fat. (3) If time points were measured for excretion (urine, feces), please provide the data.

Conclusion:

This study is inadequately reported and is considered invalid until supporting data is received. All supporting experimental results for all animals for all time points is needed, as mentioned above.

C.F. Chaisson

John Brantner, Ph.D.
TOXICOLOGY BRANCH/HED
TS-769

END